

## WHAT IS CLAIMED:

1. A thermostable ligase having 100 fold higher fidelity than T4 ligase and 6 fold higher fidelity than wild-type *Thermus thermophilus* ligase, when sealing a ligation junction between a pair of oligonucleotide probes hybridized to a target sequence where there is a mismatch with the oligonucleotide probe having its 3' end abutting the ligation junction at the base immediately adjacent the ligation junction.
2. A thermostable ligase according to claim 1, wherein said thermostable ligase has 50 fold higher fidelity than T4 ligase and 5 fold higher fidelity than wild-type *Thermus thermophilus* ligase, when sealing a ligation junction between a pair of oligonucleotide probes hybridized to a target sequence where there is a mismatch with the oligonucleotide probe having its 3' end abutting the ligation junction at the base penultimate to the ligation junction.
3. A thermostable ligase according to claim 2, wherein, in the presence of a  $Mn^{2+}$  cofactor, said thermostable ligase has a 12 fold higher fidelity than wild-type *Thermus thermophilus* ligase, when sealing a ligation junction between a pair of oligonucleotide probes hybridized to a target sequence where there is a mismatch with the oligonucleotide probe having its 3' end abutting the ligation junction at the base immediately adjacent to the ligation junction.
4. A thermostable ligase according to claim 3, wherein the thermostable ligase has an arginine adjacent its active site lysine in the KXDG motif where X is any amino acid.
5. A thermostable ligase according to claim 1, wherein, in the presence of a  $Mn^{2+}$  cofactor, said thermostable ligase has a 12 fold higher fidelity than wild-type *Thermus thermophilus* ligase, when sealing a ligation junction between a pair of oligonucleotide probes hybridized to a target sequence where there is a

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mismatch with the oligonucleotide probe having its 3' end abutting the ligation junction at the base immediately adjacent to the ligation junction.

6. A thermostable ligase according to claim 5, wherein the  
5 thermostable ligase has an arginine adjacent its active site lysine in the KXDG motif where X is any amino acid.

7. A thermostable ligase according to claim 1, wherein the  
10 thermostable ligase has an arginine adjacent its active site lysine in the KXDG motif where X is any amino acid.

8. A thermostable ligase according to claim 1, wherein the  
15 thermostable ligase has a molecular weight of 78 to 81 kDa determined by SDS-PAGE.

9. A thermostable ligase according to claim 1, wherein the  
thermostable ligase has an amino acid sequence of SEQ. ID. No. 1.

10. A thermostable ligase having 50 fold higher fidelity than T4  
20 ligase and 5 fold higher fidelity than wild-type *Thermus thermophilus* ligase, when sealing a ligation junction between a pair of oligonucleotide probes hybridized to a target sequence where there is a mismatch with the oligonucleotide probe having its 3' end abutting the ligation junction at the base penultimate to the ligation junction.

11. A thermostable ligase according to claim 10, wherein, in the  
25 presence of a  $Mn^{2+}$  cofactor, said thermostable ligase has a 12 fold higher fidelity than wild-type *Thermus thermophilus* ligase, when sealing a ligation junction between a pair of oligonucleotide probes hybridized to a target sequence where there is a mismatch with the oligonucleotide probe having its 3' end abutting the ligation  
30 junction at the base immediately adjacent to the ligation junction.

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12. A thermostable ligase according to claim 11, wherein the thermostable ligase has an arginine adjacent its active site lysine in the KXDG motif where X is any amino acid.

5 13. A thermostable ligase according to claim 10, wherein the thermostable ligase has an arginine adjacent its active site lysine in the KXDG motif where X is any amino acid.

10 14. A thermostable ligase having an arginine adjacent its active KXDG motif where X is any amino acid.

15 15. A thermostable ligase having, in the presence of a  $Mn^{2+}$  cofactor, a 12 fold higher fidelity than wild-type *Thermus thermophilus* ligase, when sealing a ligation junction between a pair of oligonucleotide probes hybridized to a target sequence where there is a mismatch with the oligonucleotide probe having its 3' end abutting the ligation junction at the base immediately adjacent to the ligation junction.

20 16. An isolated DNA molecule encoding a thermostable ligase, wherein the thermostable ligase has a 100 fold higher fidelity than T4 ligase and 6 fold higher fidelity than wild-type *Thermus thermophilus* ligase, when sealing a ligation junction between a pair of oligonucleotide probes hybridized to a target sequence where there is a mismatch with the oligonucleotide probe having its 3' end abutting the ligation junction at the base immediately adjacent the ligation junction.

25 17. An isolated DNA molecule according to claim 16, wherein said thermostable ligase has 50 fold higher fidelity than T4 ligase and 5 fold higher fidelity than wild-type *Thermus thermophilus* ligase, when sealing a ligation junction between a pair of oligonucleotide probes hybridized to a target sequence where there is a mismatch with the oligonucleotide probe having its 3' end abutting the ligation junction at the base penultimate to the ligation junction.

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18. An isolated DNA molecule according to claim 16, wherein, in the presence of a  $Mn^{2+}$  cofactor, said thermostable ligase has a 12 fold higher fidelity than wild-type *Thermus thermophilus* ligase, when sealing a ligation junction between a pair of oligonucleotide probes hybridized to a target sequence where there is a mismatch with the oligonucleotide probe having its 3' end abutting the ligation junction at the base immediately adjacent to the ligation junction.

19. An isolated DNA molecule according to claim 16, wherein the thermostable ligase has an arginine adjacent its active site lysine in the KXDG motif where X is any amino acid.

20. An isolated DNA molecule according to claim 16, wherein said thermostable ligase has a molecular weight of 78 to 81 kDa determined by SDS-PAGE.

21. An isolated DNA molecule according to claim 16, wherein the thermostable ligase has an amino acid sequence of SEQ. ID. No. 1.

22. An isolated DNA molecule according to claim 16, wherein said DNA molecule has a nucleotide sequence of SEQ. ID. No. 2.

23. An isolated DNA molecule according to claim 16, wherein said DNA molecule hybridizes to a nucleic acid having SEQ. ID. No. 2 under stringent conditions.

24. A DNA expression system transduced with a heterologous DNA molecule according to claim 16.

25. A DNA expression system transduced with a heterologous DNA molecule according to claim 17.

26. A DNA expression system transduced with a heterologous DNA molecule according to claim 18.

27. A DNA expression system transduced with a heterologous DNA molecule according to claim 19.

28. A host cell transduced with a heterologous DNA molecule according to claim 16.

29. A host cell transduced with a heterologous DNA molecule according to claim 17.

30. A host cell transduced with a heterologous DNA molecule according to claim 18.

31. A host cell transduced with a heterologous DNA molecule according to claim 30.

32. A method for detecting, in a sample, a target nucleotide sequence which differs from other nucleotide sequences in the sample by one or more single base changes, insertions, deletions, or translocations, said method comprising:

providing a sample potentially containing a target nucleotide sequence which differs from other nucleotide sequences in the sample by one or more single base changes, insertions, deletions, or translocations;

providing one or more oligonucleotide probe sets, each characterized by (a) a first oligonucleotide probe having a target specific portion and (b) a second oligonucleotide probe having a target-specific portion, wherein the oligonucleotide probes in a particular set are suitable for hybridization to a target nucleotide sequence which differs from other nucleotide sequences in the sample by one or more single base changes, insertions, deletions, or translocations and for ligation together when hybridized adjacent to one another on the target nucleotide

sequence, but have a mismatch which interferes with such ligation when hybridized to any other nucleotide sequence present in the sample;

providing a thermostable ligase having 100 fold higher fidelity than T4 ligase and 6 fold higher fidelity than wild-type *Thermus thermophilus* ligase, when sealing a ligation junction between a pair of oligonucleotide probes hybridized to a target sequence where there is a mismatch with the oligonucleotide probe having its 3' end abutting the ligation junction at the base immediately adjacent the ligation junction;

blending the sample, the one or more oligonucleotide probe sets, and the thermostable ligase to form a ligase detection reaction mixture;

subjecting the ligase detection reaction mixture to one or more ligase detection reaction cycles comprising a denaturation treatment, wherein any hybridized oligonucleotides are separated from the target nucleotide sequence, and a hybridization treatment, wherein the oligonucleotide probe sets hybridize at adjacent positions in a base specific manner to their respective target nucleotide sequences, if present in the sample, and ligate to one another to form a ligation product sequence containing the target specific portions connected together with the ligation product sequences for each set being distinguishable from other nucleic acids in the ligase detection reaction mixture, wherein the oligonucleotide probe sets may hybridize to a nucleotide sequence in the sample other than their respective target nucleotide sequences but do not ligate together due to a presence of one or more mismatches and individually separate during the denaturation treatment; and

detecting the presence of ligation product sequences produced as a result of the target nucleotide sequence being present in the sample.

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33. A method according to claim 32, wherein said thermostable ligase has 50 fold higher fidelity than T4 ligase and 5 fold higher fidelity than wild-type *Thermus thermophilus* ligase, when sealing a ligation junction between a pair of oligonucleotide probes hybridized to a target sequence where there is a mismatch with the oligonucleotide probe having its 3' end abutting the ligation junction at the base penultimate to the ligation junction.

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34. A method according to claim 33, wherein, in the presence of a  $Mn^{2+}$  cofactor, said thermostable ligase has a 12 fold higher fidelity than wild-type *Thermus thermophilus* ligase, when sealing a ligation junction between a pair of oligonucleotide probes hybridized to a target sequence where there is a mismatch with the oligonucleotide probe having its 3' end abutting the ligation junction at the base immediately adjacent to the ligation junction.

35. A method according to claim 32, wherein, in the presence of a  $Mn^{2+}$  cofactor, said thermostable ligase has a 12 fold higher fidelity than wild-type *Thermus thermophilus* ligase, when sealing a ligation junction between a pair of oligonucleotide probes hybridized to a target sequence where there is a mismatch with the oligonucleotide probe having its 3' end abutting the ligation junction at the base immediately adjacent to the ligation junction.

36. A method according to claim 32, wherein the thermostable ligase has an arginine adjacent its active site lysine in the KXDG motif where X is any amino acid.

37. A method according to claim 32, wherein the thermostable ligase has a molecular weight of 78 to 81 kDa as determined by SDS-PAGE.

38. A method according to claim 32, wherein the thermostable ligase has an amino acid sequence of SEQ. ID. No. 1.

39. A method according to claim 32 further comprising:  
amplifying, prior to said blending, the target nucleotide sequence present in the sample.

40. A method according to claim 39, wherein said amplifying is carried out by polymerase chain reaction.

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41. A method for detecting, in a sample, a target double stranded nucleic acid formed from first and second complementary target nucleotide sequences which differ from other nucleotide sequences by one or more single base changes, insertions, deletions, or translocations, said method comprising:

5 providing a sample potentially containing a target double stranded nucleic acid formed from first and second complementary nucleotide sequences which differs from other nucleotide sequences in the sample by one or more single base changes, insertions, deletions, or translocations;

10 providing a first oligonucleotide probe set, characterized by (a) a first oligonucleotide probe having a target specific portion and (b) a second oligonucleotide probe having a target-specific portion, wherein the oligonucleotide probes in the first set are complementary to the first target nucleotide sequence which differs from other nucleotide sequences in the sample by one or more single base changes, insertions, deletions, or translocations and are suitable for ligation together  
15 when hybridized adjacent to one another on the first target nucleotide sequence, but have a mismatch which interferes with such ligation when hybridized to any other nucleotide sequence present in the sample;

20 providing a second oligonucleotide probe set, characterized by (a) a third oligonucleotide probe having a target specific portion and (b) a fourth oligonucleotide probe having a target-specific portion, wherein the oligonucleotide probes in the second set are complementary to the second target nucleotide sequence which differs from other nucleotide sequences in the sample by one or more single base changes, insertions, deletions, or translocations and are suitable for ligation together when hybridized adjacent to one another on the second target nucleotide  
25 sequence, but have a mismatch which interferes with such ligation when hybridized to any other nucleotide sequence present in the sample;

30 providing a thermostable ligase having 100 fold higher fidelity than T4 ligase and 6 fold higher fidelity than wild-type *Thermus thermophilus* ligase, when sealing a ligation junction between a pair of oligonucleotide probes hybridized to a target sequence where there is a mismatch with the oligonucleotide probe having its 3' end abutting the ligation junction at the base immediately adjacent the ligation junction;



blending the sample, the first and second oligonucleotide probe sets, and the thermostable ligase to form a ligase chain reaction mixture;

subjecting the ligase chain reaction mixture to one or more ligase chain reaction cycles comprising a denaturation treatment, wherein any

5 hybridized oligonucleotides are separated from the target nucleotide sequences, and a hybridization treatment, wherein the oligonucleotide probe sets hybridize at adjacent positions in a base specific manner to their respective target nucleotide sequences, if present in the sample, and ligate to one another to form a ligation product sequence containing the target specific portions connected together with the ligation product sequences for each set being distinguishable from other nucleic acids in the ligase  
10 chain reaction mixture, wherein the oligonucleotide probe sets may hybridize to a nucleotide sequences in the sample other than their respective target nucleotide sequences but do not ligate together due to a presence of one or more mismatches and individually separate during the denaturation treatment; and

15 detecting the presence of ligation product sequences produced as a result of the target nucleotide sequence being present in the sample.

42. A method according to claim 41, wherein said thermostable ligase has 50 fold higher fidelity than T4 ligase and 5 fold higher fidelity than wild-type *Thermus thermophilus* ligase, when sealing a ligation junction between a pair of  
20 oligonucleotide probes hybridized to a target sequence where there is a mismatch with the oligonucleotide probe having its 3' end abutting the ligation junction at the base penultimate to the ligation junction.

25 43. A method according to claim 42, wherein, in the presence of a  $Mn^{2+}$  cofactor, said thermostable ligase has a 12 fold higher fidelity than wild-type *Thermus thermophilus* ligase, when sealing a ligation junction between a pair of oligonucleotide probes hybridized to a target sequence where there is a mismatch with the oligonucleotide probe having its 3' end abutting the ligation junction at the base  
30 immediately adjacent to the ligation junction.

44. A method according to claim 41, wherein, in the presence of a  $Mn^{2+}$  cofactor, said thermostable ligase has a 12 fold higher fidelity than wild-type *Thermus thermophilus* ligase, when sealing a ligation junction between a pair of oligonucleotide probes hybridized to a target sequence where there is a mismatch with the oligonucleotide probe having its 3' end abutting the ligation junction at the base immediately adjacent to the ligation junction.

45. A method according to claim 42, wherein the thermostable ligase has an arginine adjacent its active site lysine in the KXDG motif where X is any amino acid.

46. A method according to claim 41, wherein the thermostable ligase has a molecular weight of 78 to 81 kDa as determined by SDS-PAGE.

47. A method according to claim 41, wherein the thermostable ligase has an amino acid sequence of SEQ. ID. No. 1.

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